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D.1 Introduction

The purpose of this appendix is to provide examples of the types of injuries that have been documented for a number of natural resources and habitats in association with incidents involving oil. Although such injuries may result from the actual discharge of oil as well as from response-related actions, this appendix only addresses the former. The natural resources discussed include:

- **Physical Natural Resources** (surface water, ground water, sediments/soils, and air)

- **Biological Natural Resources - Species Groups**

  - Birds
  - Marine Mammals
  - Freshwater and Terrestrial Mammals
  - Reptiles and Amphibians
  - Fish
  - Shellfish

- **Biological Natural Resources - Habitats**

  - Emergent Wetlands
  - Submerged Aquatic Vegetation
  - Coral Reef Ecosystems
  - Shoreline Communities
  - Benthic Ecosystems
  - Terrestrial Ecosystems

Each section includes a brief summary of the sensitivity of the natural resource or habitat to oil, a listing of indicators of exposure and examples of the types of measurement methods used to document exposure, a description of the methods commonly used for injury determination, and a list of references where trustees can find additional information. The natural resources and habitats discussed in this appendix are not meant to be all inclusive. On-going research continues to expand our knowledge of how oil affects these and other natural resources and habitats. The literature cited in this appendix will continue to expand as new information is generated.

D.2 Physical Natural Resources

D.2.a Sensitivity to Oil Impacts

Physical natural resources include surface water, ground water, sediments and soils, and air. These resources often are the primary pathway of exposure to oil. This section addresses direct injuries that affect these resources, usually in the form of contamination at levels that impair services provided to other natural resources and/or humans.

Surface water is the physical resource most often affected by oil because spilled oil frequently reaches a water body. Most crude oils and refined products have a low water solubility, less than 100 mg/L and usually less than 50 mg/L (Sutton and Caulder, 1975; McAuliffe, 1987). The most water-soluble components in oil are also the most volatile, so evaporation as well as dilution rapidly reduce the amount of oil dissolved in water. Incidents on land seldom contaminate ground water, primarily because the high viscosity of most oils limits penetration into surface sediments. Underground discharges from buried tanks and pipelines can affect ground water, with the largest spread of contamination most often resulting from discharges of light refined products such as gasoline. For NRDAs involving oil spills, contamination of ground water is treated as a pathway to other natural resources and habitats, rather than a natural resource in and of itself.

Sediments and soils often are contaminated during incidents, primarily as a result of direct contact with the oil such as at the water/shoreline interface for floating oil. Subaqueous sediments are at risk under specific conditions (see discussion in section on Benthic Ecosystems). Response efforts are seldom effective at removing all sediment contamination, particularly where removal activities pose a high risk of further injury, such as on mud flats.

Non-petroleum compounds in crude oils, such as metals, are seldom of environmental concern for sediment contamination. For example, after the discharge of an estimated 160 to 340 million gallons of crude oil during the 1991 Gulf War, trace metal concentrations in oiled intertidal and subtidal sediments were not above background levels (Fowler et al., 1993). Spills from crude oil pipelines, however, can contain high salinity water, which can adversely affect freshwater and terrestrial resources. Refined products may contain toxic, non-petroleum additives.

Injury to air during incidents involving oil is rarely addressed. Evaporation of oil is considered to be a desirable weathering process removing the lighter, more toxic fractions from the water and soils. Recently there has been concern about benzene exposures to response personnel early during an incident, because of the chemical's classification as a human carcinogen. Overexposure is possible under the right conditions (Eley et al., 1989) namely volatile oil, low wind, restricted spreading, and sheltered areas where the vapors can pocket. A large incident near a populated area could raise health concerns for the general public, from either volatilization or combustion by-products. Particulates from the combustion of oil, those less than 10 microns (PM-10), pose the greatest risk to the respiratory tract (Wright, 1978).

D.2.b Indicators of Exposure

Indicator of Exposure	Measurement Methods
Petroleum hydrocarbon content	Sampling and laboratory analysis of air, water, and/or sediments/soils to quantify the amount of oil contamination, fingerprint the oil, and characterize oil weathering.
Petroleum hydrocarbon by-product content	Sampling of air, water, and/or sediments/soils to quantify the amount of oil by-products. For air, combustion by-products would be of greatest concern. For water, intermediate oxidation by-products would be of concern because they are highly water soluble and have acute toxicity.

Total Petroleum Hydrocarbons, PAH, and Oxidation by-Products in Water. Petroleum hydrocarbons in water can be measured using ultraviolet fluorescence (UV/F), infrared spectrometry (IR), and gas chromatography using USEPA Methods 418.1 and 8015, or American Society for Testing and Materials (ASTM) Methods D 3414, 3415, and 3650. Individual and total PAHs in water can be quantified by Gas Chromatography/Mass Spectroscopy (GC/MS) (IOC, 1991). Ehrhardt and Burns (1993) and Burns (1993) describe new methods for quantification of oxidation by-products, but few laboratories have experience with these methods.

Total Petroleum Hydrocarbons and PAH in Sediments. Total extractable hydrocarbons in sediments and soils can be measured gravimetrically after extraction (USEPA Method 503) or by UV/F (USEPA Method 418.1). Samples with high biogenic hydrocarbon content need additional cleanup steps during the extraction process or they may have high detection levels. Individual and total PAHs in sediments can be quantified by GC/MS (IOC, 1991).

Fingerprinting of oil involves a complex series of chemical and interpretative techniques that increase the confidence with which the source of oil in the sample can be inferred (McAuliffe et al., 1988; Sauer and Boehm, 1991). The confidence in the ability to fingerprint the discharged oil decreases with time (due to weathering) and distance (due to the potential for contamination from other sources of petroleum hydrocarbons). Both aliphatic and aromatic hydrocarbons are used to confirm the presence of petroleum and for fingerprinting.

D.2.c Injury

Injuries to physical natural resources are primarily determined by measurement of toxicity or violation of established standards. Use of established standards is limited because there are very few standards for specific petroleum hydrocarbon compounds in the various media, and those that do exist are mainly for pyrogenic hydrocarbon compounds that comprise only small amounts of typical oils.

Water and Sediment Toxicity Measures. There are two approaches used to characterize the toxicity of water and sediments:

- Direct measurement of the biological response of a test organism placed in water or sediment from the discharge site; and

- Comparison of the level of the contaminants in the sample, as determined by chemical analysis, with levels of contamination known to cause adverse effects (e.g., acute and chronic toxicity testing).

Direct measurement can be in-situ, for example, transplanting of infauna to contaminated sediments. Measurement may also involve the collection of sediments or water for controlled toxicity tests in the laboratory. In-situ methods can be complicated by the presence of other sources of toxicity not related to the discharge in the media being tested. Laboratory tests are designed for testing of a specific contaminant, but may not be realistic in terms of the level, pathway, and duration of actual exposures. Standard tests have been published for water and sediment for many different fish and invertebrates (ASTM, 1992; PSEP, 1991; USEPA, 1985), echinoderm sperm cell fertilization (Dinnel *et al.*, 1987), and bacteria (PSEP, 1991). The advantages and disadvantages of toxicity testing are summarized in Chapter 3.

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D.3 Biological Natural Resources - Species Groups

D.3.a Birds

D.3.a.1 Sensitivity to Oil Impacts

Many field and laboratory studies have demonstrated the differences in the effects of oil on various groups of birds. The three most important factors affecting sensitivity are behavior, distribution, and reproductive rate. Two indices have been developed to quantify the factors influencing the vulnerability of each species, the Oil Vulnerability Index of King and Sanger (1979), and the Bird Oil Index of Wahl et al. (1981). These indices and other literature were used to generate the following relative sensitivity rankings for each group of species, with emphasis on marine birds. This information is less relevant for terrestrial species, however the same principles can be used to assess the sensitivity of birds to terrestrial conditions. Note that these rankings are general guidelines. Actual conditions will likely dictate how birds are affected by a specific incident.

<u>Highly Sensitive Bird Groups</u>

Diving Pelagic Seabirds (Alcids)

- Alcids are considered to be the most vulnerable of all bird groups to oil. They form large flocks and spend most of the time floating on cold, offshore waters. For incidents in their habitats, alcids usually comprise the largest fraction of birds directly killed by oil.

- Large-scale mortality of eggs is likely because alcids form large breeding colonies in open marine settings.

- There can be long-term impacts on reproduction because of irregular cycles in breeding success, nesting abandonment and mate switching by oiled adults (Fry et al., 1987), various effects on eggs and chicks ultimately leading to lower survival rates, lower prey availability, and social disruptions at colonies that affects timing and success of egg-laying (Nysewander et al., 1993).

Waterfowl (Diving ducks, dabbling ducks, brant)

- Direct mortality from exposure to floating slicks can be high, especially during incidents involving persistent oils and when large numbers of birds are concentrated in migration and overwintering areas. For most coastal incidents, diving ducks are at greatest risk because of their preference for nearshore marine waters. In comparison, dabbling ducks prefer shallow, freshwater habitats with a reduced risk of an incident (RPI, 1988).

- Direct mortality of oiled eggs can occur but is less frequent because adults and nests are dispersed during the breeding season.

- Oiled but surviving birds often experience behavioral and physiological problems that leads to reduced reproduction from abandoned nesting activities (Hartung, 1965), reduced courtship behavior (Holmes et al., 1978), and disrupted egg-laying and incubation cycles (Holmes, 1984). These responses can result from oil ingestion during preening of oiled plumage.

- Reproductive failure can also result from ingestion of oil-contaminated prey, especially for those species (e.g., harlequin ducks) that feed primarily on intertidal invertebrates (Patten, 1993).

Diving Coastal Birds (Pelicans, loons, grebes, cormorants, boobies)

- Direct mortality from contact with floating slicks can be high because these birds regularly roost in moderate-sized flocks on nearshore coastal waters and they dive into the water to feed.

- Colonial nesting species (pelicans, cormorants, boobies) are more vulnerable than non-colonial nesters because they concentrate in breeding colonies.

Moderately Sensitive Bird Groups

Diving Pelagic Seabirds (Albatrosses, petrels, fulmars, shearwaters, skuas, jaegers)

- These birds are extremely reliant on open-water marine habitats for feeding and roosting, making them susceptible to incidents in these settings. They scatter over large areas, however, they may congregate in large rafts.

- There have been numerous studies documenting many reproductive effects for seabirds from external oiling and oil ingestion, including colony abandonment and mate switching (Fry et al., 1987), reduced laying and incubation of eggs (Fry et al., 1986), egg and chick rejection and desertion (Butler et al., 1988), and low chick growth rates (Trivelpiece et al., 1984).

Shorebirds (Sandpipers, plovers, turnstones, phalaropes)

- Direct mortality rates are generally low for shorebirds because they spend very little time in the water. Phalaropes are the exception because they winter on the open ocean where they behave more like diving pelagic seabirds.

- Sublethal effects from either reduced or contaminated prey are more likely for shorebirds because they feed in intertidal habitats where oil strands and persists. For species which form very large migrating flocks, loss of critical forage areas during migration could cause high mortalities.

Raptors (Bald eagles, osprey, peregrine falcons)

- Raptors become oiled primarily via consumption of oiled prey, particularly eagles and falcons that may take oiled, disabled birds.

- Reproductive failures can be caused by oiling of eggs as well as disturbance from shoreline cleanup operations (Bowman and Schempf, 1993).

Less Sensitive Bird Groups

Wading Birds (Herons, egrets, rails)

- Direct mortality of wading birds is usually low because they wade in shallow, sheltered waters to feed. However, their plumage can become contaminated by walking through oiled vegetation.

- Indirect effects on reproduction can occur from loss of prey, causing hatchling starvation, particularly for species unable to shift to alternative foraging sites (Parsons, 1990; 1991).

Gull and Terns

- These species are usually oiled in low proportion to the exposed populations because they are readily able to avoid oil. Gulls in particular are highly adaptable, opportunistic feeders, and prolific breeders.

D.3.a.2 Indicators of Exposure

Birds may be directly exposed to oil through oiling of plumage and eggs, ingestion of oil during preening, ingestion of oiled prey, absorption, and inhalation of oil through the skin or egg. The following methods can be used to document exposure:

Indicator of Exposure	Measurement Methods
Direct oiling of plumage/skin	Visual estimates of number of individuals or percent of flock/study group by degree of oil coverage on plumage; photographic or video documentation; sampling of oiled feathers to fingerprint and characterize oil weathering.
Direct oiling of eggs	Counts of percent of eggs oiled; samples to fingerprint and characterize oil weathering.
Oil ingestion	Discharged oil in stomach contents and/or feces to document actual oil ingestion, even months or years post-spill. Oil and/or metabolites in bird tissues to document the degree and duration of exposure. Oil in preferred prey items can be used to confirm the source and estimate duration of oil exposure.
Tissue damage	Post-mortem examination of lung tissue for hemorrhagic lesions from inhalation of oil vapors, and of other internal organs for lesions from inhalation of oil vapors.

D.3.a.3 Injury

In addition to the direct pathways of exposure listed above, birds may be indirectly affected by oil through habitat loss (e.g., vegetation mortality), habitat degradation, and diminished food populations. Commonly used methods for injury determination are discussed below.

Acute Mortality. Rehabilitation centers keep records on numbers of recovered dead and surviving birds, by species, sex, and age. These data, corrected for the background number of dead birds, provide the minimum count of birds affected by the incident. To expand the count, trained observers can survey shorelines to conduct carcass counts. Survey methods are provided in Ford et al. (1987) for marine species and Fite et al. (1988) for terrestrial species. Following these guidelines can improve the accuracy of these mortality estimates. Otherwise, problems such as insufficient or incomparable data for beach carcasses throughout the study area or over time can increase the uncertainty in the mortality estimate. Only persons with a Federal permit are allowed to collect or conduct experiments on migratory or endangered birds.

Simple extrapolations can be used to estimate total mortality from the carcass counts. There are also computer models that use currents, wind, bird distributions, beached bird counts, and other factors during the incident to estimate total number of dead birds (Ford et al., 1991). High natural variability in bird distributions, both spatially and seasonally, makes it difficult to estimate the total and exposed population actually present during an incident.

Recovered birds can be examined to determine cause of death and document exposure to the oil. Methods include collection of samples of oiled plumage and gut contents to fingerprint oil, blood and tissue analysis for oil residues, and histological analysis of tissues to determine cause of death and to rule out other non-incident related causes of death (Leighton, 1995).

Reduced Reproduction. There are many measures of reproductive success that can be used to assess injury such as number of nests built, clutch size, egg-laying dates, hatching success/growth rates, and fledgling success. Field studies usually compare rates for exposed and reference nesting colonies. This approach works best when there is extensive knowledge of the normal rates or behavior for the study population or species, such as in Parsons (1990, 1991) where oil-affected colonies were part of a five-year study on nesting and foraging ecology prior to the incident.

Laboratory studies may be used to document reduced reproduction for the oil type or degree of weathering (e.g., Stubblefield et al., 1993), particularly when direct observation of reproductive behavior is not possible (such as oiled waterfowl that dispersed to remote nesting sites).

There can be many causes of reduced reproductive success including loss of nesting habitat, disruption of courtship, incubation, attention, and feeding patterns and social structures, loss of prey, and toxicity from oil coating or ingestion of contaminated food. It is important to understand the cause of an observed reduction in reproduction in order to link the incident and the observed effect. Birds can experience total nesting failure on a regular basis, making it difficult to determine oil-related injury.

Reduced Survival. Sublethal impacts associated with exposure to oil or indirect effects can reduce the overall survival rates of birds. Banding of oiled birds released after rehabilitation can be used to document survival and reproductive rates. Studies of feeding behavior patterns can show longer time spent feeding or longer distances traveled because of loss of prey and degradation of foraging habitat (Parsons, 1990).

These studies often include chemical and histopathological analysis of tissues from exposed birds, such as PAH levels in tissues and elevated mixed function oxygenase (MFO) activity in the liver (Gorsline and Holmes, 1982) to document on-going exposures, and liver, kidney, and intestinal necrosis to document physiological responses to exposure that could lead to reduced survival (Fry and Lowenstine, 1985).

Habitat Loss or Degradation. Because birds rely heavily on wetlands and aquatic prey, habitat loss and degradation are extremely important to local populations. Methods to quantify habitat loss or degradation are discussed in section B.4.

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D.3.b Marine Mammals

D.3.b.1 Sensitivity to Oil Impacts

Most marine mammals have special management status as threatened or endangered species. A brief summary of their sensitivity to oil by groups is provided below.

Baleen Whales. These whales have a series of elongated, bristled structures (baleen) in the mouth acting as filters to separate food items (mostly small crustaceans and fish) from seawater. Laboratory studies have not found any evidence that oil or tarballs significantly foul the feeding apparatus of baleen whales, and whale skin is nearly impermeable, even to the most volatile oil fractions (Geraci, 1990). Baleen whales, however, are considered to be the most vulnerable to oil discharges, based on their generally low numbers, feeding strategies (skimming the surface and scouring of the bottom) that increase the risk of oil ingestion, and dependence on specific sites for feeding and reproduction (Würsig, 1990).

Toothed Whales and Dolphins. These cetaceans capture individual prey items using toothed jaws. Most prey is captured below the water surface so there is little risk of direct ingestion of floating oil during feeding. Most species are highly mobile and wide-ranging, except for belugas and narwhals. Following the *Exxon Valdez* incident, fourteen killer whales were lost from a very stable pod from 1989 through 1991. The seven deaths that occurred immediately may have resulted from inhalation of volatile gases or oil ingestion, six more deaths that occurred within one year after the incident may have resulted from residual effects or consumption of contaminated prey (Dahlheim and Matkin, 1993). Dolphins can see oil on the surface and can avoid it (Geraci, 1990; Sumltea and Würsig, 1992), thus they are not considered to be particularly sensitive to oil discharges.

Fur Seals. These seals rely on dense fur as the primary means of insulation and thermoregulation. Fouling of one-third of the body surface resulted in a 50 percent increase in heat loss in fur seals (Kooyman et al., 1976). Thus, they are susceptible to death by hypothermia and stress. Other known effects of oil include ingestion-related mortalities, interference with swimming ability, lethargic behavior, irritation of the respiratory system from inhalation of fumes, and inflammation of mucous membranes (St. Aubin, 1990).

Other Seals and Sea Lions. These animals rely on a thick layer of blubber for insulation. Pinnipeds other than fur seals are less threatened by thermal effects of fouling (St. Aubin, 1990). Young animals with fur would be at greatest risk. Direct oiling of animals and their haulouts can cause mortality, as well as internal damage. Frost and Lowry (1993) reported debilitating lesions in the brains of harbor seals taken from oiled areas following the *Exxon Valdez* incident. Conditions that would lead to the highest mortality include exposure of animals early and close to the discharge, heavy contamination around haulouts, and sub-populations already stressed by disease or limiting environmental conditions (St. Aubin, 1990).

Walruses and Polar Bears. These two very different species are grouped together because both are associated with pack ice, and little is known about how oil affects them. Walruses are highly gregarious and form large non-breeding haulouts. They have sparsely distributed hair, so thermal stress is not likely to be important (St. Aubin, 1990). In contrast, polar bears occur in low densities as solitary animals or family groups. However, they must maintain a clean pelt for thermoregulation, and would likely undergo thermal stress if oiled. Polar bears have been shown to ingest oil during grooming (Stirling, 1990).

Manatees. Little information is available regarding the effects of oil exposure on manatees. Manatees are considered able to detect and avoid oil (St. Aubin and Lounsbury, 1990). They tend to concentrate in shallow water, increasing the risk of direct contact with oil. Their non-selective feeding habits may allow them to consume floating tarballs along with their normal foods. If a discharge were to occur in their preferred habitat during winter, manatees may be forced into colder waters inducing thermal stress. Displacement during summer months would not be as disturbing (St. Aubin and Lounsbury, 1990).

Suspected injury to manatees could include irritation to mucous membranes and lungs, dermal membrane irritation, interference with gastric gland secretions, and loss of intestinal flora (Geraci and St. Aubin, 1980). Increased boat activity during response efforts could also result in manatee injury or death.

Sea Otters. Sea otters are highly sensitive to oil because they have dense fur for thermoregulation, groom excessively (ingesting oil); have a metabolism rate so high that they must consume 23 to 33 percent of their body weight per day, consume benthic organisms that tend to accumulate petroleum hydrocarbons, form large concentrations in coastal areas, with high site fidelity, and spend much time in kelp beds that tend to trap and hold oil (Ralls and Siniff, 1990).

D.3.b.2 Indicators of Exposure

Marine mammals may be directly affected by uptake of oil via the water surface, while grooming and from ingestion of food. Indicators of exposure and measurement methods are listed below:

Indicator of Exposure	Measurement Methods
Direct oiling of skin/fur	Visual estimates of number of individuals or percent of study group by degree of oil coverage on body surface; photographic or video documentation. Sampling of oiled materials to fingerprint and characterize oil weathering.
Oiling of habitat	Maps of oil distribution on the water surface and in preferred habitats using standardized methods and descriptors (Owens and Sergy, 1994). Sampling of oiled materials to fingerprint and characterize oil weathering.
Oil ingestion	Discharged oil in stomach contents and/or feces to document actual oil ingestion. Oil in tissues to document the degree and duration of exposure. Visual observations of animals consuming oiled prey.
Tissue damage	Post-mortem examination of lung tissue for hemorrhagic lesions from inhalation of oil vapors and of other internal organs for lesions from inhalation of oil vapors.
Increased mixed function oxygenase (MFO) activity	Tissue samples collected from fresh specimens and analyzed for hepatic cytochrome P4501A (Payne et al., 1986). Marine mammals appear to have the liver enzymes needed to metabolize and excrete petroleum hydrocarbons. Although there is no systematic dose-response relationship, laboratory and field studies have found an increase in MFO following oil exposure (Geraci and St. Aubin, 1990).

D.3.b.3 Injury

In addition to direct effects from contact with discharged oil, marine mammals may be indirectly affected by oil through habitat degradation (particularly contaminated haulout areas) and diminished prey populations. Injury determination methods for marine mammals are summarized below. Only a limited number of laboratory studies on a very small number of individuals have been conducted to confirm cause and effects of petroleum exposures. Many sublethal injuries have been suspected based on knowledge of life history and ecology of marine mammals. The size and behavior of most marine mammals precludes capture-based study methods, thus most studies have to be conducted using visual observation and census techniques.

Mortality. Mortality investigations are conducted by aerial, boat, and foot surveys to identify and count dead organisms, usually shortly after the discharge. Because of their large size, most stranded marine mammals (except sea otters) are readily sighted, so mortality estimates may be lower due to carcasses sinking. Only persons with a Federal permit are allowed to conduct work on marine mammals, thus all sightings should be reported to the Marine Mammal Stranding Network. Trained mammalogists can collect the necessary data, photographs, and samples for necropsy to confirm cause of death and chemical samples for fingerprinting. Early reporting of carcasses is very important because tissues break down rapidly.

A second approach is to compare post-discharge counts with pre-discharge data, using the same or similar survey methods to increase the validity of the comparisons. High seasonal variations and incomplete pre-discharge coverage for the affected area/populations can be serious limitations. This approach is best used for stable, well-studied populations.

A third approach is to develop computer models to simulate oil movement, the distribution and abundance of animals, and the likelihood of intersection between the two. Such an intersection model was developed to estimate sea otter mortality following the *Exxon Valdez* incident (Bodkin and Udevitz, 1993).

Reduced Reproduction. Reproductive impacts are determined by monitoring for the number and survival of young. Marine mammals nurture their young for periods ranging from one month to two years, thus it is possible to observe and count parents and young over time to determine survival rates. Photo-identification techniques have been used to identify and track individual whales in stable pods according to their unique markings (Bigg et al., 1986). However, there is often a lack of baseline data on life history (birth rates, survival rates for juveniles and adults, etc.) for many species and sub-populations.

Reduced Survival. Sublethal effects of exposure can eventually lead to reduced survival. Behavioral effects (e.g., lethargy, reduction in feeding effort, increased vulnerability to predation) can be noted during observations of oiled and unoiled populations, so that oil-related responses can be differentiated from normal behavior. Reduced growth rates can be measured, but sample sizes are usually small, making data interpretation more difficult.

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D.3.c Freshwater and Terrestrial Mammals

D.3.c.1 Sensitivity to Oil Impacts

Freshwater mammals at risk from oil-related injuries include river otter, beaver, mink, nutria, and muskrat. Like sea otters, these animals spend much of the time in water, have high site fidelity, and rely on fur to maintain thermoregulation. They are highly susceptible to direct mortality. Terrestrial mammals of concern include species associated with water bodies and riparian habitats, such as bear, panther, moose, fox, deer, and raccoon. These species are likely to be affected by the consumption of oiled food items as well as by direct contact and habitat degradation.

Little is known about the impacts of oil on freshwater and terrestrial mammals. Acute effects from contamination of fur and ingestion of oil during preening and chronic effects from ingestion of contaminated food are most likely. In oiled/reference area comparisons of river otters in *Exxon Valdez* studies, researchers found a less diverse diet, lower body mass, larger home ranges, avoidance of preferred habitat, and abnormal blood characteristics in animals from oiled areas one year after the incident (Bowyer et al., 1993). Efforts were made to determine differences in populations for oiled/control study areas, but the confidence limits for the population estimates overlapped for most surveys. A laboratory study to determine the influence of hydrocarbons on reproduction in ranched mink was planned, but never conducted. Thus, there are little data on whether sublethal doses of oil will influence reproduction in terrestrial mammals.

Field studies also were conducted to determine effects of the *Exxon Valdez* incident on Sitka black-tailed deer, which concentrate on beaches during late winter and early spring to forage on intertidal marine vegetation. Study plans included comparisons of the number of dead deer on oiled versus reference islands and the hydrocarbon levels in tissues and rumen contents, however, the study results have not been published.

D.3.c.2 Indicators of Exposure

Freshwater and terrestrial mammals may be directly affected by contact with oil on the water surface and oiled vegetation while grooming and from contaminated food. Indicators of exposure and measurement methods are listed below:

Indicator of Exposure	Measurement Methods
Direct oiling of fur	Visual estimates of number of individuals or percent of study group by degree of oil coverage on body surface; photographic or video documentation; sampling of oiled fur to fingerprint and characterize oil weathering.
Oiling of habitat	Maps of the distribution of oil on the water surface and in preferred habitats using standardized methods and descriptors (Owens and Sergy, 1994); sampling of oiled materials to fingerprint and characterize oil weathering.
Oil ingestion	Discharged oil in stomach contents and/or feces to document actual oil ingestion. Oil in tissues to document the degree and duration of exposure.
Tissue damage	Post-mortem examination of lung tissue for hemorrhagic lesions from inhalation of oil vapors, and of other internal organs for lesions from inhalation of oil vapors.

D.3.c.3 Injury

In addition to direct effects from contact with or ingestion of discharged oil, freshwater and terrestrial mammals may be indirectly affected by oil through habitat degradation and diminished food availability. Injury determination methods are summarized below.

Mortality. Surveys of the affected areas to count the number of animals killed (body count) by the incident typically include systematic methods using transects or quadrats to count/collect dead or oiled animals (Anderson et al., 1976). The total number of animals killed are extrapolated from the sampled data, using actual mortality rates for the known survey area modified with correction factors to account for differences between the surveyed area and the entire impact zone. Small mammals, such as oiled beach mice, are likely to be quickly scavenged by predators or return to their burrows thereby avoiding discovery by survey teams. Thus, these counts may underestimate the actual number of animals killed. However, field surveys are important in documenting that exposure and mortality have occurred to each species of concern.

If there are other likely causes of mortality for the species of concern, it may be important to determine the cause of death in a representative number of animals. Other possible causes could include a large winter kill or high incidence of disease. Dead animals from the oiled area can be collected for necropsy and histopathological analysis for comparison with animals collected from outside the oiled areas.

For species with very limited populations, it may be possible to estimate changes in population based on the estimated mortality. Otherwise, studies of population densities between oiled and control areas may be used. The actual field methods for detecting population density changes would be selected based on the behavioral characteristics of each species and availability of historical population distribution data. Measurement of significant differences between impacted and reference sub-populations, particularly for larger animals with low densities and long lifetimes, is extremely difficult, although there are standard methods in use for data collection and analysis (e.g., Davis and Winstead, 1980; Seber, 1982; Shirley et al., 1988; Chao, 1989; Pollock et al., 1989).

Reduced Reproduction. For most incidents, it may be difficult to directly measure reproductive success in wild populations of small mammals. There is a general lack of baseline data on life history (birth rates, survival rates for juveniles and adults, etc.) for many species and sub-populations. Reproductive injury can be assessed by investigation of the reproductive potential through study of physiological effects on the reproductive organs. Such studies could include comparisons of the histology of the gonads of males and females in the oiled and control populations; or the size, development, and contents of the uterus of mature females can be used to determine if gonadal failure is evident.

Alternatively, it may be preferable to conduct laboratory studies to assess the influence of oil on reproduction. If sublethal effects on reproduction are thought to be significant for a species, laboratory experiments may be used to demonstrate a direct cause and effect relationship between exposure and changes in reproduction, in support of field observations of such changes. Otherwise, because of the limited data on the effects of oil on reproductive performance in freshwater and terrestrial mammals, it may be difficult to prove that the oil exposure was the cause of the observed changes. In developing laboratory experiments, it is important to ensure that the oil used in the experiments is the same product that was discharged and has weathered to the same degree as the oil to which wild animals have been exposed.

Reduced Survival. Sublethal impacts associated with exposure to oil or indirect effects can reduce the overall survival rates of exposed animals and/or populations. Tagging of oiled animals released after rehabilitation can be used to document survival and reproductive rates of oiled/cleaned individuals, usually the smaller species such as river otters or beaver. In the field, behavioral effects (e.g., lethargy, reduction in feeding effort, increased vulnerability to predation) are recorded during observations of oiled and unoiled populations, so that oil-related effects can be quantified. Reduced growth rates or body mass can be measured, but usually sample sizes are small, making data interpretation more difficult.

Indirect effects can be caused by reductions in available food or having to shift to less-productive habitats. Studies of food habits, movements, and habitat selection can show longer time spent feeding or longer distances traveled because of degradation of foraging habitat. Study of feces can document differences in the diet in oiled versus unoiled areas, supporting other observations of reduced viability.

These studies can include chemical and histopathological analysis of tissues from exposed animals to document on-going exposures, and liver, kidney, and intestinal necrosis to document injury. Bowyer et al. (1993) monitored specific blood parameters in oiled and unoiled populations of river otters, using the results to indicate exposure and some degree of physiological injury. These measurements support the weight of evidence by documenting pathways, exposures, and biological responses that can be used to estimate a reduction in the overall viability of the exposed population.

Habitat Degradation. There are various biological indicators of habitat degradation appropriate to assessment of injuries to freshwater and terrestrial mammals. Two possible indicators include changes in food habits and habitat use. Changes in food habits can result from both contamination or localized reductions in preferred food items. Food habits can be described from prey remains in feces or examination of the stomach contents of collected animals. Habitat-use studies are more complex, consisting of descriptions of activity patterns (e.g., percent time spent foraging and resting), distances traveled to foraging areas or home range size, and other factors appropriate to the species. Methods to assess these indicators include time and area-constrained observations during which records of the percent time spent on various activities are recorded.

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D.3.d Reptiles and Amphibians

D.3.d.1 Sensitivity to Oil Impacts

Reptiles and amphibians are a complex group of organisms, with highly diverse life histories, physiologies, survival strategies, and habitat requirements. The species at greatest risk from an incident are those associated with open marine, estuarine, and riverine habitats such as sea turtles, crocodiles, and alligators. Other wetland-associated species are at moderate risk and terrestrial species are at lowest risk. There are many threatened and endangered species of reptiles and amphibians in freshwater habitats that could be at risk from an incident in these areas.

Because of their diversity, it is not possible to predict the relative sensitivity among species groups. There are little data on effects of petroleum hydrocarbons on reptiles and amphibians, with the exception of sea turtles. Hall and Henry (1992) found that it was not possible to extrapolate study results from other vertebrate classes (mostly fish) for even general conclusions on the relative toxicity of chemicals. Because of these limitations, most assessment studies of oil impacts to reptiles and amphibians have focused on counting the number of dead animals.

The effects of oil are best known for sea turtles, because of their status as threatened/ endangered and because of their higher risk of exposure from marine incidents. The direct and indirect effects of oil on sea turtles can be divided into three general categories based on the life stage and habitat affected by the oil:

- Direct effects on eggs and hatchlings on nesting beaches;
- Direct effects on hatchlings, juvenile, and adult turtles at sea; and
- Indirect effects resulting from impacts to turtle habitats both in the water and on the beach.

Direct Effects on Eggs and Hatchlings by Stranded Oil. Various researchers have studied the physiological and behavioral effects of oil on each life stage in laboratory experiments (Fritts and McGehee, 1982; Vargo et al., 1986; Lutz et al., 1986). The major conclusions on the effects of oil on eggs and hatchlings from these studies are summarized below.

- The number of unhatched eggs in a nest was much higher when fresh crude oil was on the surface of the sand during the last half or quarter of incubation, due to displacement of oxygen by the lighter oil fractions when the rate of oxygen consumption in the nest is at its peak.

- Weathered crude oil was less toxic to turtle eggs than fresh crude oil.

- Hatchling morphology was affected by the amount and time of oiling.

Studies by Mahaney (1994) on frogs found no effect of crankcase oil on hatching success, but no successful metamorphosis of highly exposed tadpoles.

Direct Effects of Oil on Juvenile/Adult Turtles at Sea. Juvenile and adult turtles are likely to contact oil slicks during the early stages of an incident and tarballs as the oil weathers. From laboratory studies on the physiological effects of oil on subadult loggerhead turtles (Lutz et al., 1986; Bossart et al., 1993), the direct effects of oil exposure include coating of sensory organs, reddening and sloughing off of the skin, dysfunction of the salt gland, uptake of oil in the gastrointestinal system, and disturbed diving and respiration patterns. Although there have been many incidents in areas populated by turtles, it is unusual to have large numbers of turtles directly affected by an incident of oil. Reports of adverse effects of oil on adult and juvenile turtles are mostly anecdotal and poorly documented as to the cause of death (Rytzler and Sterrer, 1970; Delikat, 1980; Hooper, 1981; Gitschlag, 1992). It is difficult to document the number of turtles affected by an incident and it is likely that many of the affected turtles may never be seen by rescue workers. High-risk areas include migratory routes, foraging areas, and areas offshore of heavily utilized nesting beaches.

The effects of pelagic tar on sea turtles have been well documented (Witham, 1978, 1983; Vargo et al., 1986; Van Vleet and Pauly, 1987; Gramentz, 1988). Turtles feed on objects floating at the water surface, therefore they are susceptible to ingestion of tar balls, which can block the oral cavity and digestive tract. Floating tar can coat the flippers, the mouth can become coated as the turtle attempts to clean its flippers. Large quantities of tar have been known to immobilize smaller turtles. Southeastern Florida has high concentrations of pelagic tar and Van Vleet and Pauly (1987) concluded that tarballs from tanker incidents were having a significant effect on turtle populations.

Indirect Effects as a Result of Impacts to Habitats. Degradation of nesting, foraging, resting, or critical habitats may have long-term effects on reptile and amphibian populations. These concerns are important in developed areas where these critical habitats are subject to many other sources of contamination, particularly for threatened and endangered species.

D.3.d.2 Indicators of Exposure

Reptiles and amphibians may be directly affected by oil through oiling of skin and eggs, ingestion of oil and oiled food, and inhalation of oil fumes. Indicators of exposure and measurement methods are listed below:

Indicator of Exposure	Measurement Methods
Direct oiling of skin and eggs	Visual estimates of number of individuals or percent of study population by degree of oil coverage on skin; photographic or video documentation; counts of percent of eggs oiled; samples of oiled eggs or oil from dead animals to fingerprint and characterize oil weathering.
Extent and degree of oil contamination of habitats	Aerial and ground surveys to make systematic, visual estimates of the areal extent and degree of oil of habitats using standardized methods and terminology (Owens and Sergy, 1994); photographic or video documentation of visual observations; sampling of oiled water and sediments to fingerprint the oil and characterize oil weathering.
Oil ingestion	Discharged oil around mouth parts, in stomach contents and/or feces to document actual oil ingestion. Oil in tissues to document the degree and duration of exposure. Oil in preferred food items to confirm the source, degree, and duration of oil ingestion.
Tissue damage	Post-mortem examination of lung tissue for hemorrhagic lesions from inhalation of oil vapors and of other internal organs for lesions from inhalation of oil vapors.

D.3.d.3 Injury

Reptiles and amphibians may be indirectly affected by oil through habitat loss (e.g., vegetation mortality), habitat degradation, and diminished prey populations. Injury determination methods for reptiles and amphibians are summarized below. Methods for assessment of sea turtles are better established than methods for other species. Survey methods for counting the number of dead animals on land and in wetlands would be similar to those listed for freshwater and terrestrial mammals (See B.3.3). Little is known about the effects of oil on most species of reptiles and amphibians; therefore, research would be needed to document the link between exposure and sublethal injuries.

Mortality. Surveys can be conducted to document dead or moribund animals on land and in the water. All oiled turtles should be reported to the Marine Mammal Stranding Network, which include sea turtles found dead in the water or onshore or alive but in a weakened condition. Under Federal law, only permitted individuals are allowed to handle sea turtles or other endangered and threatened animals.

Quantification of the number of oiled turtles at sea is more difficult. It is likely that oiled animals will be difficult to observe from aircraft. As demonstrated during at-sea capture efforts for turtles at the *Mega Borg* incident in the Gulf of Mexico, it is very difficult to capture healthy adult turtles at sea (Gitschlag, 1992). Therefore, only seriously injured or trapped turtles are likely to be captured.

It may be important to determine the cause of death through histopathological analysis (Van Vleet et al., 1986) although this can difficult in old specimens.

Reduced Reproduction. Except for sea turtles, there is little information on the likely effects of oil exposure on reproductive potential of reptiles and amphibians. Site-specific studies of exposed populations would be needed to document reproductive effects on these animals. The high genetic variability in amphibians needs to be considered in any study design.

For sea turtles, monitoring of oiled and reference nests can be conducted to compare hatching success, emergence success, etc. with degree and nature of oil contamination. If all nests cannot be monitored, a stratified-random sampling strategy can be used to select nests for monitoring. Maps of oiled nesting beaches and nest counts can be used to extrapolate the total impact on nesting success. Selected samples of addled eggs and dead hatchlings can be examined to determine cause of mortality. Lights used for night cleanup activities could cause disorientation and reduced survival of hatchlings.

Reduced Survival. Sublethal impacts resulting from exposure to oil or indirect effects could reduce the overall survival rates of exposed animals, but there are few existing studies that predict these effects. Documentation of reduced survival might have to be accomplished through detailed studies of exposed populations, even for sea turtles.

Habitat Degradation. When incidents have occurred in habitats known to be highly utilized by the species of concern for foraging or resting, studies can be conducted to determine the extent and degree of habitat degradation. Conditions when such impacts might occur include heavy oil that eventually sinks, contaminating benthic habitats, light, refined products that result in mortality to preferred food items that are sensitive to oil or high-wave energy conditions that naturally disperse a light crude oil or refined product in shallow waters, causing mortality and oil accumulation in benthic invertebrates and sediment contamination. Oil residues and cleanup activities can degrade important habitats for threatened and endangered reptiles and amphibians, particularly in wetlands.

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D.3.e Fish

D.3.e.1 Sensitivity to Oil Impacts

The probability of adverse changes to fish from oil is influenced by the inherent sensitivity and susceptibility of each species, duration of exposure, and temperature. Sensitivity and susceptibility are functions of life history stage and habitat preference, behavior, diet, and other factors. Each life stage has characteristics that directly control the likelihood and degree of impact from an incident (RPI, 1987) as summarized below.

The sensitivity of fish eggs is high, but lower than the larval stage due to the presence of protective membranes that may reduce exposure of the developing embryo to oil. Susceptibility of eggs is highly variable. Benthic eggs released in deep water are unlikely to be exposed to floating oil during an incident. Benthic eggs released in shallow waters are vulnerable to exposure to light oils having a significant water-soluble fraction, non-floating oils, and dispersed oil. Benthic eggs spawned on intertidal or very shallow subtidal substrates are highly vulnerable to direct mortality from contact with floating slicks, the water-accommodated oil fraction, and contaminated sediments.

The larval stages of most marine fish are planktonic, their large-scale movements are controlled by water currents. Within the first few days or weeks, planktonic larvae start feeding on phytoplankton and zooplankton, which are concentrated in the upper water column. Larval life stages are the most sensitive to acutely toxic effects of oil because of their preference for the upper water column and shallow, estuarine habitats.

Adult fish are considered to be the least sensitive life stage to oil impacts because they are highly motile and better able to detect and avoid discharges, have fully developed dermal protection, and have a metabolic capability to degrade oil. Acute toxicity is most likely to occur when light, refined products are spilled in shallow, confined waterbodies or in creeks and small rivers where the entire waterbody can be contaminated (Vandermeulen, 1987). Territorial fish also are highly susceptible. At the *Morris J. Berman* incident in Puerto Rico, for example, the heavy oil sank in nearshore lagoons and territorial fish in the lagoons experienced high mortality and sublethal effects (Vicente, 1994). Chronic impacts are of greater concern for species that use shallow, nearshore habitats because these habitats are most likely to be contaminated by oil. After chronic exposure to oiled sediments, benthic fish have been shown to exhibit reduced feeding, growth, and reproduction, as well as histopathological changes (Haensly et al., 1982; McCain et al., 1978; Collier et al., 1993). There could be long-term, sublethal injuries where subtidal sediments in nursery areas have been contaminated. Historically, extensive subtidal sediment contamination with measurable fishery injuries have been documented for very few incidents, with the *Amoco Cadiz, Exxon Valdez,* and *Braer* as notable exceptions.

Recent laboratory research on the toxicity of the degradation by-products of petroleum hydrocarbons has shown that these by-products have high acute toxicities to fish and that the toxicity is increased when microbes and nutrients are used to speed degradation (Doug Middaugh, USEPA, pers. comm.). Studies by Burns (1993) of bivalve tissue from beaches heavily oiled by the *Exxon Valdez* incident showed that a complex assemblage of intermediate hydrocarbon oxidation by-products were bioavailable for uptake in marine organisms several years post-spill. Thus, oxidation by-products may be an additional source of chronic exposure and effects on fish populations.

D.3.e.2 Indicators of Exposure

Direct measurement of petroleum hydrocarbons in fish tissue may not always be an appropriate indicator of exposure because of the high rate of metabolism of petroleum by most fish species (Varanasi et al., 1989). Methods have been developed, however, to detect exposure by measurements of petroleum metabolites, which are rapidly excreted through the bile, or by measuring increases in mixed function oxygenase (MFO) enzymes. The presence of fluorescent aromatic carbon (FAC) in the bile, for example, is evidence of a relatively recent exposure to oil. Although there is no systematic dose-response relationship, there are many laboratory and field studies showing an increase in MFO activity following oil exposure (Collier et al., 1993). Petroleum metabolites in bile, however, cannot be used to identify the source of the oil exposure. Indications of exposure are listed below:

Indicator of Exposure	**Measurement Methods**
Petroleum hydrocarbon metabolites in bile	Bile collected from freshly caught fish to measure the fluorescent aromatic carbon (FAC) content by fluorescence spectroscopy (Krahn et al., 1992).
Increased MFO activity	Tissue samples collected from live fish and analyzed for hepatic cytochrome P450 (Payne et al., 1986).
Tissue damage	Fish (moribund or from affected habitats) preserved for histological examination (Meyer and Barclay, 1990; Huggett et al., 1992).

D.3.e.3 Injury

Fish may be directly affected by uptake of oil via water, contaminated sediments, and food. They may be indirectly affected by oil through habitat loss (e.g., dieback of seagrass beds in nursery areas), habitat degradation, and diminished prey populations. Injury assessment methods for fish are summarized below.

Mortality. Fish-kill surveys estimate the number of adult fish killed immediately after an incident. Although the American Fisheries Society (AFS, 1992) and U.S. Fish and Wildlife Service/USFWS (Meyer and Barclay, 1990) have recently updated their publications on fish-kill methods, these approaches often greatly underestimate the total injuries from an incident because they only estimate the number of dead adult fish. Fish-kill investigations are more appropriate in streams and small rivers where the entire water surface along the sampling transect can be surveyed and the dead fish tend to accumulate within a reasonable distance from their original habitat. The method can be augmented with snorkeling surveys to detect and count dead fish that sink.

Reduced Abundance and Diversity. Changes in the number of fish or species resulting from an incident can be measured by comparing pre- and post-incident abundances at the same sites, or paired oiled and unoiled sites where pre-incident data are not available and the paired sites are comparable (Hilborn, 1993). The value of pre- versus post-incident surveys in quantifying oil-related injuries to fish will depend on natural variability in the measured parameters, reliability of the data-collection methods, and degree of injury caused by the incident. For many species, the year-to-year variability is so large that only severe impacts could be measured at statistically significant levels. Prior to developing study plans for quantification of population-level injuries using this method, the degree of change that would have to occur from pre- to post-incident in order to be statistically different should be estimated and the reasonableness of that level of change should be evaluated. Also, recent natural events (e.g., cold weather, droughts, hurricanes) should be evaluated with respect to their potential for confounding changes for a particular incident.

Oiled versus unoiled comparisons have similar limitations, with the added difficulty of finding truly representative reference sites. Sampling plans should include analyses of the likely variability in the data and the number of replicates needed to increase the statistical power of the comparisons to a level needed to detect a minimum change.

Abundances can be measured using standard fisheries survey techniques, including diver counts along transects, trawls and tows, counting of anadromous fish at weirs in streams, and tagging and marking of fish. Rapid bioassessment techniques such as those USEPA developed for rapid fish surveys in streams and rivers (Plafkin et al., 1989) are useful as quick screening tools to determine if there is a need for more detailed, quantitative surveys.

Where population-level changes are difficult to measure directly, a biological-effects model in conjunction with a population model can be used. Biological effects are derived from exposure levels estimated from a physical fates or water quality model for the incident conditions and toxicity test data (either from the literature or using local communities and the discharged material). Exposure concentrations and conditions are used to calculate mortality rates and sublethal effects. These effects are then applied to data on species abundance and structure to quantify impacts. The DOI Type A models (NRDAM/CME and NRDAM/GLE) uses this approach to calculate the mortality and lost weight of both adult and larval fish resulting from exposure to toxic fractions of the oil during a discharge, as well as reduced recruitment and lost productivity (French and Reed, 1993).

Reduced Reproduction. Study methods to measure reduced reproduction under both laboratory and field conditions include reduced egg viability and hatchability (Rice et al., 1983; McGurk and Biggs, 1993) and larval malformations (Hose et al., 1993).

Reduced Survival. Sublethal impacts associated with exposure to oil or indirect effects can reduce the overall survival rates of fish. A wide range of behavioral responses to oil exposure have been investigated in laboratory studies, including avoidance/preference (Rice, 1985), reduced locomotor activity and predator avoidance (Berge et al., 1983), changes in feeding activity (Williams and Kiceniuk, 1987), disruption of chemoreception and homing signals (Nakatani and Nevissi, 1991), and reduced growth and altered respiration rates (Rice et al., 1983). Histopathological analysis of tissues (Huggett et al., 1992) from exposed fish can be used to document physiological responses to exposure that could lead to reduced survival, including fin rot, lesions on the liver, kidney, spleen, gills, and olfactory nares, and tumors. These measurements support a weight of evidence approach by documenting pathways, exposures, and biological responses that can be used to estimate a reduction in the overall viability of the exposed population.

Fish Tainting. Although fish usually metabolize petroleum hydrocarbons, tissue concentrations can reach levels where consumption poses a health risk or tainting affects taste and/or smell. Although there are no food safety standards specifying a maximum contaminant level for oil or petroleum hydrocarbons in seafood, guidelines followed in the past state that if the seafood tastes or smells oily, it is not safe to eat. Tainting is as much a perception problem as a real risk. Fear of tainting can result in a loss of a natural resource service as serious as actual tainting.

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D.3.f Shellfish

D.3.f.1 Sensitivity to Oil Impacts

Shellfish are grouped into crustaceans (e.g., shrimp, lobster, and crab), molluscs (e.g., abalone, oyster, clam, mussel, scallop, gastropod, and chiton), and cephalopods (e.g., squid and octopus). There have been numerous studies on the toxicity, uptake, and depuration of petroleum hydrocarbons for shellfish (compiled in Scott et al., 1984). The effects of exposure to oil are influenced by the inherent sensitivity and susceptibility of the species and are a function of their life-history stage, habitat preference, behavior, and diet. Each stage has characteristics that directly control the likelihood and degree of impact during an incident (RPI, 1989).

In general, life stage sensitivity to oil impacts decreases from the egg to the adult life stages (Scott et al., 1984). However, life cycle circumstances make larvae more likely (i.e., more vulnerable) than eggs to be injured by oil. For many shellfish species, the eggs are either benthic or nektonic, reducing their vulnerability to floating slicks. There are notable exceptions, such as white shrimp that can spawn in shallow water and near the surface. However, the larvae of most species are found near the water surface in shallow, estuarine water bodies, making them highly vulnerable to oil. Juveniles and adults occupy similar habitats and have similar vulnerabilities to oil.

Bivalve molluscs and shrimp lack the ability to metabolize petroleum hydrocarbons, thus they readily accumulate these compounds in their tissues. Once the source of exposure is removed, however, depuration can occur within a few days to months. For example, following dispersion of No. 6 fuel oil in shallow, nearshore waters, oysters attached to rocky substrate in 4-6 m water depth were sampled one and four weeks post-incident, and targeted PAHs dropped by 94-98 percent over the three-week period (Michel and Henry, 1994). Bioaccumulation is influenced by the lipid content of the organism, which can change according to its reproductive status. Contaminated molluscs can provide a pathway for exposure of other natural resources that feed heavily on them.

Observations of discharges of heavy oil have shown that crabs can be directly exposed when the oil sinks. Their mouth parts typically become heavily oiled from feeding on tarballs. Laboratory studies have shown that hydrocarbon uptake with food by crabs does not accumulate but is eliminated in the feces (Lee et al., 1976).

D.3.f.2 Indicators of Exposure

Because shellfish are filter feeders or grazers and generally do not metabolize petroleum hydrocarbons, measurement of body burdens is very appropriate for documentation of oil exposure.

Indicator of Exposure	Measurement Methods
Direct oiling of external parts	Visual observations of oil on beds or individual animals; photographic or video documentation; oil samples for fingerprinting.
Petroleum hydrocarbons in tissue	Analysis of tissues from live organisms for petroleum hydrocarbon content and fingerprinting the oil to identify its source.
Tissue damage	Specimens from affected habitats preserved for histological examination (Huggett et al., 1992).

D.3.f.3 Injury

The physiological indicators of stress in shellfish, especially molluscs, have been well documented (Scott et al., 1984). Sublethal effects of oil exposure include depressed feeding, changes in respiration rates (both decreases and increases), reduced growth, decreased gonadal condition, tissue necrosis, and behavioral changes such as decreased burrowing and slower tactile response (Scott et al., 1984). Injury assessment methods for shellfish are summarized below.

Mortality. Shellfish may be directly and acutely killed by coming into contact with toxic levels of oil in the water column or being smothered by oil stranded on intertidal shellfish beds. Quantification of the number of individuals or percent of a specific population killed is accomplished in a variety of approaches, depending upon the species type, habitat, and life stage affected. Concentrations of juvenile and adult animals in the water column are usually too patchy for trawling to be of value. Quantification of impacts to planktonic life stages are often difficult to detect, but plankton tows can be used to observe whether post-larvae are generally normal or moribund. Pots can be used for benthic species (lobsters, crabs, large shrimp) to detect differences in abundance in comparable oiled and unoiled areas. Viability of eggs on gravid females can be measured on captured animals.

Where affected animals are stranded onshore (e.g., the *North Cape* oil spill in Rhode Island where large numbers of moribund lobsters and surf clams were washed ashore), systematic counts of the number of stranded animals using quadrats and/or transects can provide the basis for calculating the minimum mortality.

For infaunal species such as clams and scallops, changes in density and abundance can be estimated from sediment cores, though large numbers of cores may be needed to provide statistically significant results. Mortality to epifaunal species, such as oysters and mussels, can be assessed through direct count, using quadrats or transects.

Reduced Abundance and Diversity. Changes in the number of species resulting from an incident can be measured by comparing pre- and post-incident abundance at the same sites, or paired oiled and unoiled sites where pre-incident data are not available and the paired sites are comparable (Hilborn, 1993). Abundance is measured using standard shellfish survey techniques such as trawls and tows for shrimp, capture in pots for crabs and lobsters, benthic cores for clams and scallops, and surface quadrat/transect counts for abalone, oysters, mussels, gastropods, and chitons.

Where population-level changes are difficult to measure directly, a biological-effects model in conjunction with a population model may be used. Biological effects are derived from exposures estimated from a physical fates or water quality model for the incident conditions and toxicity test data (either from the literature or using local communities and the discharged material). Exposure concentrations and conditions are used to calculate mortality rates and sublethal effects. These effects are then applied to data on species abundance and structure to quantify impacts. The DOI Type A model uses this approach to calculate the mortality and lost weight for shellfish resulting from exposure to toxic fractions of the oil, as well as reduced recruitment and lost productivity (French and Reed, 1993).

Reduced Reproduction. Study methods to measure reduced reproduction under both laboratory and field conditions include reduced egg viability and hatchability (Rice et al., 1983), reduced spawn settlement rates, and changes in spawning patterns (Houghton et al., 1992).

Shellfish Tainting. Because many types of shellfish bioaccumulate petroleum hydrocarbons, tissue contaminations can reach levels where consumption poses a health risk or tainting affects taste and/or smell. Although there are no food safety standards specifying a maximum contaminant level for oil or petroleum hydrocarbons in seafood, guidelines followed in the past state that if the seafood tastes or smells oily, it is not safe to eat. Tainting is as much a perception problem as a real risk. Fear of tainting can result in loss of natural resource services as serious as actual tainting.

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D.4 Biological Natural Resources - Habitats

D.4.a Wetlands

D.4.a.1 Sensitivity to Oil Impacts

The wetlands habitat includes salt and fresh water wetlands of all types, namely mangroves and marshes, forested swamps, floating vegetation, and wet tundra. Most information regarding the impact of oil on wetlands comes from studies of oil impacts on the vegetation of coastal tidal estuaries. Most of the studies have been of marshes dominated by *Spartina alterniflora* and mangroves dominated by *Rhizophora mangle.* Based on the available data, there are significant differences in the degree of impact of oil among species assemblages. Although every incident is unique, there are several factors that affect the behavior and impact of oil on wetlands.

Impacts by Oil Type. Light refined products (e.g., No. 2 fuel oil) have the greatest acute toxicity to all types of vegetation, but have shorter persistence and a lower likelihood of sediment contamination. For herbaceous vegetation, crude oils and intermediate refined products show mostly medium-term impacts, a higher tendency for sediment contamination, and recovery within 1 to 5 years (Alexander and Webb, 1983; Baca et al., 1983; Bender et al., 1980; Michel, 1989). In contrast, heavy crude and refined oils can severely affect mangroves, where toxicity results from coating of the roots which prevents gas exchange (Getter et al., 1984). Impacts can last up to 20 years, until the mangrove returns to a mature forest habitat.

Extent of Vegetation Contamination. Many plants can survive partial oiling. Few survive when all or most of the above-ground vegetation is coated (Alexander and Webb, 1983; 1985; Baker, 1971).

Degree of Sediment Contamination. The degree of contamination of sediments can prolong impacts to marsh ecosystems for many years, compared with the initial loss of oiled vegetation. Slower recolonization rates are frequently related to hydrocarbon levels in the sediments, though the composition of the oil is as important as the total petroleum content (Alexander and Webb, 1987). Studies of a large incident in Panama showed that chronic oiling from oil slowly released from sediments had significantly reduced recolonization by mangrove prop root communities for five years (Burns et al., 1993).

Physical Setting. The relative degree of exposure to waves and currents is one of the most important factors controlling the persistence of oiled vegetation and overall rate of recovery. Exposure to waves and currents usually works to enhance recovery, but in some cases it can also work to increase erosion after plant roots die and before new growth can occur. Many isolated freshwater wetlands have little or no exposure to physical removal processes and are thus susceptible to long-term oil persistence and effects.

Seasonal Effects. The timing of an incident relative to the growing season can affect the nature and duration of the impact. In general, oiling during the dormant season has the lowest impact, whereas oiling of vegetation during the growing season will likely have longer effects (Baker, 1971). Mechanisms responsible for the slower recoveries during the growing season have not been extensively studied, but probably are related to plant stress and food reserves at a time when the plant's resources are being fully expended (Mendelssohn, 1993, personal communication).

Species Sensitivity. Although there are limited data, annuals may be more sensitive than perennials because annuals have small root systems and low food reserves, whereas perennials are able to regenerate from underground rhizomes (Baker, 1979). However, annuals tend to be the first recolonizers (Getter et al., 1984).

Effects of Cleanup. During response activities, the vegetation and substrate can be trampled and the oil can get mixed deeper into the substrate, extending the injuries both in degree and duration. Trustees also need to protect vegetation from trampling during injury assessment studies.

D.4.a.2 Indicators of Exposure

Exposure can be documented through both visual and chemical measures. The extent and degree of oiling on vegetation and in the substrate are important variables in quantification of the injury. Indicators of exposure are listed below:

Indicator of Exposure	Measurement Methods
Extent and degree of oil contamination on vegetation and substrate, and disturbance by trampling	Aerial and ground surveys for systematic, visual estimates of the areal extent and degree of oil adhering to vegetation and substrate; photographic/video documentation, using standardized terminology (Owens and Sergy, 1994); summary statistics on the total acreage of injured wetland habitat by degree of oiling and trampling categories.
Contamination of biotic and abiotic components	Collection of samples for chemical analysis to measure petroleum hydrocarbons levels; to identify the source of the contamination; and to characterize the degree of weathering.

D.4.a.3 Injury

Injury assessment studies of past incidents have concentrated primarily on injury to vegetation. An injury assessment should generate data on severity of injury, total acreage of injured wetland, and duration of the injury. It is important that field study designs consider the effect that other environmental factors may have on plant recovery including changes in salinity (Winfield and Mendelssohn, 1994), water level, or temperature. Detailed field methods for assessing oiled marshes are provided in Mendelssohn et al. (1990) and Michel et al. (1994). Field methods for study of oiled mangroves are provided in Getter et al. (1981), Getter and Ballou (1985), Garrity et al. (1994), and Levings and Garrity (1995). Injury assessment methods for wetlands are summarized below.

Percent Live and Dead Cover. To quantify injury to vegetation, estimates of the percent live and dead cover or trees can be made along transects or in randomly located quadrats in each category of oiling (as determined from aerial mapping). Transects can be used where it is important to consider topographic controls. Using fixed transects and quadrats allows better control for long-term monitoring of changes in cover. Ground stations can be used to verify estimates of vegetation die-back or stress measured from aerial photography.

Species Abundance and Diversity. Abundance may be recorded at the species level so that temporal changes in species composition can be monitored. Such studies are important when there is a potential for re-colonization of oiled areas by pioneering species, which might not be detected by simple live/dead cover or biomass assessment methods.

Reproductive Status or Potential. At selected sampling sites along transects or in quadrats, quantitative measurements of the reproductive status of the plants can be recorded for comparison of oiled versus reference sites. For mangroves, seedling density and condition are sensitive indicators.

Above-ground Biomass. Net above-ground effect on production is determined by counting the number and height of all stems within quadrats (Morris and Haskins, 1990). Estimates of individual stem biomass can be accomplished by harvesting the vegetation from selected quadrats within the marsh.

Observations of Effects on Marsh Fauna. It is important to make systematic observations on obvious effects on dominant marsh fauna. Observations include the presence/absence of organisms, qualitative estimates on relative abundance, visual extent of oil contamination, and behavioral observations. If a more rigorous assessment of impacts to marsh fauna is appropriate, methods to use can be found in the section on shoreline communities (B.4.4).

Net Erosion. Along exposed shorelines, there is a risk of shoreline erosion after the oiled vegetation dies back. Stakes can be placed landward of the shoreline and the distance to the shoreline measured at regular intervals (Michel et al., 1994). Only under extreme erosion conditions can shoreline changes in wetlands be detected using sequential aerial photography.

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D.4.b Submerged Aquatic Vegetation

D.4.b.1 Sensitivity to Oil Impacts

Submerged aquatic vegetation (SAV) includes rooted vascular plant species that grow primarily below the water surface in both fresh and salt water (e.g., water lilies, eel grass, surf grass, manatee grass, kelp). SAV is considered to be highly sensitive to oil impacts because of its high productivity, key role in nutrient cycling, and value as nursery, foraging, and sheltering habitats for many endangered and commercially and recreationally important species. However, SAV is not as vulnerable as intertidal vegetation because it is mostly subtidal and less likely to be in direct contact with floating oil slicks. Oil effects on SAV habitats as discussed in Zieman et al. (1984) are summarized below:

- Greatest impacts occur on SAV that is on the water surface or in the intertidal zone, where the oil comes in direct contact with exposed blades.

- Oil readily adheres to exposed blades, particularly when the oil is heavy or weathered.

- Oiled SAV quickly defoliates but the plants have the capacity to grow new leaves (the leaves grow from a relatively protected meristem) in a relatively short period of time unless the sediments also are oiled. Recovery can occur with 6-12 months.

- Plant mortality has been observed during incidents when the sediments were contaminated by oil although such incidents have been rare.

- The most sensitive component of the SAV ecosystem is the epiphytic community and juvenile organisms that utilize the grass beds as a nursery. These species and life stages can be highly sensitive to both the water-soluble and insoluble fractions of oil.

- The plants can uptake hydrocarbons from the water column and sediments, potentially lowering their tolerances to other stresses.

D.4.b.2 Indicators of Exposure

Exposure can be documented through both visual and chemical measures. Degree of oiling on vegetation and in the substrate is an important variable in quantification of the injury. Oiled seagrass blades are quickly sloughed off, so early surveys are needed to document exposure. Indicators of exposure are listed below:

Indicator of Exposure	Measurement Methods
Direct oiling of vegetation	Visual estimates of the areal extent and degree of oil on blades/leaves; photographic or video documentation; sampling of oiled vegetation to fingerprint the oil and characterize oil weathering. For kelp, maps of distribution of oil slicks in kelp beds over time.
Oil contamination in sediments and water	Collection and analysis of sediment from below and water samples from above the SAV beds. Oil stranded on adjacent shorelines may be a chronic source of exposure.

D.4.b.3 Injury

Most injury assessments focus on injury to the SAV bed itself because it is the basis for a highly productive ecosystem. An injury assessment should generate data on severity of injury, total acreage of injured SAV, and duration of the injury. Careful site selection for oiled and reference sites is particularly important for seagrass beds, to make sure that they have similar physical settings in terms of current and wave energy, substrate type, water depth, and so forth. In some cases, it may be important to demonstrate similarity of oiled and reference sites by continuing the evaluation of injury over time until natural recovery has progressed and the measured parameters converge. An excellent source for seagrass assessment methods is Phillips and McRoy (1990). Injury assessment methods for SAV are summarized below.

Biomass. Measurements of biomass can have extremely high variability, thus many replicates per site may be needed to support statistical analysis. Although the standing crop of leaves is significant, the majority of the biomass is in the rhizomes and roots, thus both above- and below-ground biomass measurements are important. Above-ground biomass can be measured by repeated clipping of the leaves (Kenworthy et al., 1993). Below-ground biomass can be measured from cores.

Species Abundance and Density. Many SAV beds follow standard successional sequences (Zieman, 1982) that result in beds dominated by a single plant species. Frequently the successional steps are reset by perturbations or environmental conditions such that the climax is not reached. Thus, relative species abundance is generally not useful in detecting oil effects. Instead, it is used to characterize the seagrass habitat in general. Relative abundance and density most frequently are measured using standard quadrat counting methods at randomly located sites. The high natural variability in SAV cover will likely require many replicates to determine differences among sites.

Growth Rates. Sublethal effects of oil exposure can result in reduced productivity and growth rates. Short-term growth of leaves can be measured by perforation with a needle at the base of shoots in quadrats and measuring growth over a time period usually of days to weeks (Thom, 1990). Eventually the leaves can be harvested to measure growth in terms of leaf area and dry weight. Long-term growth can be measured by tagging rhizomes at the base of the most recent shoot, then returning months later to collect the tagged segments and any new growth (Houghton et al., 1992). Reduction in flowering shoot density has been reported for several incidents and may be a sensitive indicator of exposure (Houghton et al., 1992; Dean et al., 1994).

Morphological Measures. Leaf area index, the ratio of leaf area to substrate surface area, provides an estimate of secondary surface area available for epibiota, habitat complexity, and photosynthetic potential (Evans, 1972). Short-shoot and leaf-pair densities may be a better indicator of biomass where there are large seasonal fluctuations in standard biomass measurements (Kenworthy, 1992).

Physiological Measures. Sub-lethal effects of oil on seagrasses can be measured by changes in the photosynthesis and respiration rates of exposed plants. Durako et al. (1993) used photosynthesis versus irradiance (i.e., radiant flux density) responses of leaf tissues exposed to oil to assess oil toxicity to seagrasses. Such laboratory experiments may be needed to link the injury to exposure for the specific oil type and seagrass species.

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D.4.c Tropical Reef Ecosystems

D.4.c.1 Sensitivity to Oil Impacts

Tropical reefs are highly productive ecosystems that experience long-term natural fluctuations as well as a wide range of responses to man-made disturbances. There have been relatively few studies of reefs following exposure to incidents involving oil. Loya and Rinkevich (1980), Ray (1980), and Tetra Tech (1982) compiled data on the effects of oil on coral reef communities for fifteen incidents. These studies looked only at acute impacts. Some sublethal work on coral reefs is documented in Fucik et al. (1984).

Long-term studies by Cubit et al. (1987), Guzman et al. (1991), and Guzman and Holst (1993) of the 1986 Texaco incident in Panama reported delayed and extensive patterns of injury to shallow coral reefs 2.5 to 5 years after the incident. The extent and degree of injury to coral reefs were related to chronic exposure as oil leached out of adjacent mangroves for years. A recent consolidation and overview of oil impacts on coral reefs was published by IPIECA (1992).

The sensitivity of coral reef ecosystems to episodic incidents can be divided into three categories:

Highly Sensitive

- Intertidal reefs and reef flats, where direct contact with the oil is likely.
- Sheltered, shallow water settings where high concentrations of dissolved and particulate oil are likely to persist.
- Areas where oil leaching from adjacent areas creates chronic oil exposures.
- Areas where coral reefs already are stressed by pollution, sedimentation, thermal problems, etc.

Moderately Sensitive

- Reefs located in water depths of 1-5 m below low water, where high levels of dissolved or particulate oil are possible, especially when the oil is fresh.
- Partially-sheltered locations where oil mixed into the water column can cause exposure for up to a few days.

Less Sensitive

- Reefs located at greater than 5 m water depth at low tide; dilution can reduce oil levels in the water column to below acute toxicity levels.

- Highly flushed settings where fresh oil could mix into the water column, but exposure is more likely to be short (hours to days).

- Healthy subtidal reefs that are likely to recover from short-term exposures within days or weeks after oil exposure.

D.4.c.2 Indicators of Exposure

Exposure can be documented through both visual and chemical measures. Oil stranded on adjacent shorelines may be a chronic source of exposure with greater long-term impacts than acute exposures during the incident event. Indicators of exposure are listed below:

Indicator of Exposure	Measurement Methods
Direct contact of reef with whole oil during low tide	Visual estimates of the areal extent and degree of oil adhering to or in direct contact with reef structure; photographic or video documentation; sampling of oiled material to fingerprint the oil and characterize oil weathering.
Direct contact with the water-accommodated fraction (both dissolved and dispersed oil)	Observations, maps, and photographs showing the presence of oil slicks in the vicinity of reefs; water samples to measure the amount of oil in the water column; computer models that calculate the water-column concentrations of oil expected in the vicinity of the reef.
Physical destruction of the reef (e.g., ship grounding)	Observations, maps, and photographs showing the extent of damage to the reef.

D.4.c.3 Injury

The focus of the injury assessment is often on the reef-building community, which is the structural basis for the reef ecosystem. It is important to note, however, that corals are not always the primary components of the tropical reef ecosystem. Calcareous red and green algae are often the dominant cover. In addition some organisms, such as sponges, may be better indicators of oil effects.

Brown and Howard (1985) review methods for assessing the effects of stress on coral reefs, many of which are applicable to injury assessment. For oil, short-term mortality is expected from physical destruction or direct exposure. Thus, the emphasis is on measures of sublethal effects that can be used to estimate the degree, areal extent, and duration of injury. It is important to document the degree and frequency of oil exposure and to stratify sampling sites according to degree and type of exposure. Injury assessment methods for coral reefs are summarized below.

Percent Cover. Quantitative methods for assessing cover can be conducted using the line-transect (point) method or the quadrat method (Weinburg, 1981). If pre-incident data are available, using the same methods as those in the previous surveys improves the strength of before-after comparisons. Fixed transects often are recommended over random ones, so that repeat surveys can confidently identify shifts in zonation. When using the point method, it is important to record what is directly under (and over, for branching corals) the point. There is a wide range in oil sensitivity among coral species that is not well known or understood.

Within the reef ecosystem, some organisms may be more abundant and at greater risk to oil impacts, such as sponges. Cover and abundance measures for these organisms should be included along the transects.

Tissue Injury Rates. Measurements of tissue injury for all sessile organisms on the reef can include lesions, necrosis, and morbidity. In general, there is a high background injury rate on reefs which should be defined. Injury categories should be objective and standardized among observers.

Growth Rate. Changes in growth rates result from a variety of physiological processes, thus growth rate can be a good indicator of oil-induced stress. However, growth rates are inherently variable among species and within a single species, requiring a large number of samples. Gladfelter et al. (1978) describe methods for measuring growth rates in the field using x-radiography for massive corals or stain markings on branching corals, as well as radioisotope dating and weighing of specimens. For sparse reefs, collecting samples for analysis can cause extensive injury to the reef. To link reduction in growth rates to health of the reef, it may be necessary to monitor direct physiological measures of injury, such as reduced reproduction.

Expulsion of Zooxanthellae. Expulsion of zooxanthellae (or bleaching) following exposure to oil has been found both in the laboratory and following spills (Birkelund et al., 1976; Neff and Anderson, 1981). Documentation of bleaching following a discharge may be evidence of short-term exposure and response.

Reproduction Rates. Guzman and Holst (1993) were able to detect reductions in gonad size of reef corals at oiled versus unoiled reefs five years after the Panama (1986) incident. They suggest that female gonads (eggs) can be the easiest method to measure changes in reproduction rates for gonochoric coral species. However, because reef sampling is destructive and sample preparation and analysis is very time-consuming and expensive, this technique is only applicable to those species for which the reproductive cycle has been previously studied. Another approach is to measure recruitment on settling plates or natural surfaces in oiled and non-oiled areas in similar habitats and time periods.

Other physiological and histopathological parameters, including mucous production, algal invasions, bacterial infections, other diseases, and reductions in metabolism, could be used to assess injury. There is little baseline information by species, however, and in general there is high natural variability in these parameters (Brown and Howard, 1985). In addition, corals exhibit these responses for a wide range of stresses that are not well understood.

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D.4.d Shoreline and Riparian Communities

D.4.d.1 Sensitivity to Oil Impacts

This grouping of shoreline communities includes all biological communities associated with shoreline and riparian habitats, including estuarine and marine intertidal zones and riverine and lacustrine shorelines, from arctic to tropical settings. Habitats include rocky shores, sand beaches, gravel beaches, tidal flats, vegetated banks, wetlands, and man-made structures. These habitats are often severely injured when oil strands on the shoreline. There have been numerous studies on the effects of oil on these habitats. Some events such as the *Torrey Canyon* incident in 1967 have been studied for over 20 years (Hawkins and Southward, 1992). Ganning et al. (1984) summarized the literature on the effects, recovery, and restoration of oiled shoreline ecosystems, mostly marine. They summarized numerous studies on acute and sublethal effects, but none on coating or habitat alterations. They concluded that it was difficult to generalize the impacts of an oil discharge because of the wide range in environmental factors controlling both the fate of the oil and community behavior. In particular, there is not a great deal of information on which to predict oil impacts to riparian habitats.

D.4.d.2 Indicators of Exposure

Exposure can be documented through both visual and chemical methods. Visual observations of the presence of oil are most important during the early phases of an incident, whereas chemical measures are valuable for documenting chronic and low-level exposures. Indicators of exposure are listed below:

Indicator of Exposure	Measurement Methods
Extent and degree of oil contamination on the substrate	Aerial and ground surveys to make systematic, visual estimates of the areal extent and degree of oil adhering to the shoreline substrate, using standardized terminology and methods (Owens and Sergy, 1994;); photographic or video documentation of visual observations; sampling of oiled substrate to fingerprint the oil and characterize oil weathering; summary statistics on the total distance of oiled shoreline by degree of oiling categories.
Sediment contamination	Collection of sediment samples for chemical analysis to measure the level and type of petroleum hydrocarbons present.
Levels of petroleum hydrocarbons in tissue	Collection of tissue samples, usually from organisms that are known to uptake and concentrate petroleum hydrocarbons, such as bivalves and gastropods.

D.4.d.3 Injury

Assessment of injury to shoreline communities is most often conducted through field measurement of population parameters and statistical analysis of the data. The primary goal is to document the community response to oiling over time by establishing enough permanent plots within the study area to quantify the changes in the measurement parameters. Study design is extremely important to being able to detect oil-related changes. It may be important to classify stations according to the degree of contamination, exposure to wave and tidal energy, habitat, elevation, and type of clean up conducted at the station. Most communities undergo complex successional stages that need to be considered in sampling design and data interpretation. Repetitive surveys should be scheduled consistently, coinciding with reproductive events or maximum development, if possible.

Another alternative is to use previously established stations (Mussel Watch, State or University long-term monitoring sites, etc.) located in the area of impacts. These sites can provide historical data on population compositions and natural variations. In addition, the Minerals Management Service is currently (1995) funding a research program to develop detailed guidelines for injury assessment studies of rocky intertidal coasts. These guidelines should have broad applicability to all shoreline habitats. Injury assessment methods for shoreline and riparian communities are summarized below.

Percent Cover and Species Abundance and Diversity Indices. Methods for measuring these community parameters are described in Littler and Littler (1985) for algae, Baker and Wolff (1987) for many different communities, Cubit and Conner (1993) for reef-flat communities, Zeh et al. (1981) and Moore and McLaughlin (1978) for intertidal communities, and Holme and McIntyre (1979) for coring of benthic fauna. Depending on the site conditions, transects are set up either parallel or perpendicular to the shoreline. Along the transects, quadrats are located either randomly or at fixed distances. Estimates of percent cover and other parameters within quadrats can be made visually or by using systematic or random point contact methods. Dethier et al. (1992) indicated that visual estimation of percent cover by experienced biologists was more accurate and precise, especially for rare species, than 50 or 100 point contact methods.

Growth Rates. Growth can be a very sensitive indicator of on-going sublethal effects on shoreline communities, either directly from contamination or indirectly from reductions in the food base. Growth is studied by collecting animals from classified sites and measuring length and/or weight at selected intervals. To improve the precision of the data, individual specimens can be tagged for re-collection and measurement. Specimens with shells can be evaluated by measuring increments between growth rings in the shell, tagging the shell chemically with a fluorescent dye (calcein) that binds with calcium, or taking repetitive measurements of shell length of individual organisms (Houghton et al., 1992). Transplanting experiments can be used to document injury and potential recovery at oiled sites (Houghton et al., 1994). For plants, growth rates can be determined by marking or tagging individual plants for repetitive length measurements over time.

Reproductive Condition. There are several methods for measuring reproduction, depending upon the species and reproductive mechanism. For species that broadcast eggs or seeds, plates can be set out to compare the settling rate in oiled versus unoiled sites. For attached plants or sedentary animals, visual estimates or counts can be made of the percent or number of the species that are in a reproductive stage.

Biomass. Nearly all methods of measuring biomass require destructive sampling, that is, all biota in a specific area are removed for analysis in the laboratory (Littler and Littler, 1985). Epifauna are scraped from the surface. Infauna can be field-sieved and preserved (Holme and McIntyre, 1979). Larger organisms can be hand-sorted, identified, and measured or weighed in the field. In the laboratory, the samples are sorted, identified to the lowest practical taxonomic level, and counted.

Species Behavior. Field observations can be made of behavior including response to tactile stimuli, gapping shells, re-attachment rates, righting ability, reactor muscle function, and so forth.

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D.4.e Benthic Ecosystems

D.4.e.1 Sensitivity to Oil Impacts

Benthic ecosystems include underwater habitats not addressed elsewhere:

- Subtidal rocky reefs and sand/mud bottoms; and

- Lake and river unvegetated bottoms.

For most incidents benthic ecosystems are usually at much less risk of significant exposure to oil. Benthic ecosystems are at risk when oil sinks, either because it is heavier than water initially, or because the oil picks up enough sediment to cause it to sink (Michel and Galt, 1995; Michel et al., 1995). Under these conditions, benthic resources can come in direct contact with heavy amounts of oil, with significant injuries.

Oil also can contaminate benthic habitats through the deposition of oil-contaminated sediments, mostly sand and mud. Extensive contamination of subtidal sediments has been documented for only the *Florida* barge in Buzzards Bay (Sanders, 1978; Sanders et al., 1980), the *Amoco Cadiz* off the coast of Brittany, France (Cabioch et al., 1982), the *Exxon Valdez* in Prince William Sound (O'Clair et al., 1993; Jewett and Dean, 1993), and the *Braer* off the Shetland Islands (Ecological Steering Group, 1993). With the exception of the *Exxon Valdez*, these incidents occurred during extremely high wave energy conditions in shallow water, where both oil and fine-grained sediments were mixed into the water column in the nearshore zone. During the *Exxon Valdez*, high-pressure washing of oil from the shoreline during the summer months probably mobilized oil and fine-grained sediment for mixing and deposition in shallow offshore areas. It appears that somewhat unique conditions are required before large-scale contamination of benthic habitats by oil is likely to occur. Muddy sediments are more likely to be contaminated than rocky reefs or even sandy bottoms where the substrate undergoes some reworking by currents and/or waves.

D.4.e.2 Indicators of Exposure

Exposure can be documented through both visual and chemical methods. Visual observations of the presence of oil in benthic habitats are difficult and feasible only under heavy oiling conditions. More commonly, samples are taken for chemical analysis or toxicity testing to document the presence of oil in these habitats. Indicators of exposure are listed below:

Indicator of Exposure	Measurement Methods
Extent and degree of oil contamination of the substrate	Sampling of sediments to quantify the amount of oil contamination, fingerprint the oil, and characterize oil weathering. Sampling methods include the use of sediment coring devices (USEPA, 1984; PSEP, 1991) or hand-held diver-collected cores.
Sediment toxicity	Collection of sediment samples for bioassays to demonstrate the presence of toxicity (Chapman, 1988). These tests provide information that is independent of chemical characterization and ecological surveys.
Levels of petroleum hydrocarbons in biota tissue	Collection of tissue samples, usually from organisms that are known to uptake and concentrate petroleum hydrocarbons, such as bivalves.

D.4.e.3 Injury

Assessment of injury to benthic ecosystems is conducted with field measurements of population parameters and statistical analysis of the data (Zeh et al., 1981). The primary goal is to document the community response to oiling over time by collecting enough samples within the study area to quantify the changes in abundance, density, diversity, and so forth. It is important to classify stations according to substrate type and degree of exposure to wave and current energy. Injury assessment methods for benthic communities are summarized below.

Mortality. Where large-scale mortality of benthic organisms is expected, divers can make observations on the extent and relative abundance of dead organisms along transects using video cameras to document these observations.

Benthic Species Abundance and Diversity Indices. Coring methods for measuring community parameters for benthic fauna are described in Holme and McIntrye (1979). Divers can census epibiota along transects using methods similar to those described for shoreline ecosystems. Rapid bioassessment techniques are useful as quick screening tools to determine if there is a need for more detailed, quantitative surveys. For example, the USEPA has published rapid bioassessment protocols for use in streams and rivers for benthic macroinvertebrates and fish (Plafkin et al., 1989).

Biomass. Infauna samples are collected from sediment grabs or dredges, field-sieved, and preserved (Holme and McIntrye, 1979). Larger organisms can be hand-sorted, identified, and measured or weighed in the field. In the laboratory, the samples are sorted, identified to the lowest practical taxonomic level, and counted.

Growth Rates. Growth is studied by collecting animals from specific locations and measuring length and/or weight at selected intervals. Specimens with shells can be evaluated by measuring increments between growth rings in the shell, tagging the shell chemically with a fluorescent dye (calcein) that binds with calcium, or taking repetitive measurements of shell length of individual organisms (Houghton et al., 1992). Transplanting experiments can be used to document injury and potential recovery at oiled sites (Houghton et al., 1994). For shoreline communities, growth rates can be determined by marking or tagging individual plants for repeat length measurements over time. For macroalgae, stipe diameter may be a good indicator of length and weight of each plant (Dean et al., 1993).

D.4.e.4 References

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D.4.f Terrestrial Ecosystems

D.4.f.1 Sensitivity to Oil Impacts

This category includes all terrestrial ecosystems, with emphasis on the most sensitive types including dry tundra, taiga, temperate grasslands, and tropical rain forests. Because of extensive development of Arctic and subarctic oil fields, there have been more studies of the effects of oil on tundra and taiga environments compared to the other types (McCown and Simpson, 1973).

Tundra and taiga soils are highly sensitive to both the physical and chemical effects of oil and to response activities (Linkins et al., 1984). Studies of experimental and accidental incidents have found extremely slow weathering rates for oil that had penetrated below the surface in arctic and subarctic soils. Slightly weathered oil was still present fifteen years after an experimental incident in taiga soils in interior Alaska (Collins et al., 1993). Three factors contribute to the long-term effects of oil in these habitats:

- Very low plant productivity and recycling of nutrients because of the short growing season, limited nutrients, and acid, organic soils;
- Slow weathering rates of stranded oil; and
- Severe access limitations, particularly in summer when physical destruction from access is unavoidable and extensive.

In general, oil impacts to terrestrial ecosystems are a function of the following factors.

Depth of Penetration. In terrestrial environments incidents usually occur as point discharges on the surface and subsurface, where penetration is a function of soil permeability; and as aerial spray, which usually causes low soil penetration. Deep penetration into soils (particularly tundra, peat and gravel soils) will likely slow the rate of weathering, and increase the duration of acute and chronic toxicity.

Potential for Temperature Change. Oil can significantly affect the soil temperature, especially in arctic and tropical settings. In arctic settings, the ground surface heat flux can be modified because albedo is decreased, leading to surface heating, solar radiation flux is increased by death of the canopy, thermal diffusivity changes because of the oil, and the organic layer is less insulative where the vegetation has died (Mackay et al., 1975). Elevated soil temperatures in arctic settings can melt permafrost, which can lead to permanent soil compaction and subsidence of the surface (Collins et al., 1993). In tropical settings, decreased albedo and die-back of the canopy can cause soil heating, dehydration, and reduced viability (Kinako, 1984).

Changes in Water-holding Capacity. One of the more important effects of oil on soils is a reduction in their water-wettability, making the soil hydrophobic (Schwendinger, 1968). Contaminated soils often resist wetting, reducing the amount of water available for uptake by plant roots.

Potential for Anaerobic Conditions. Oiled soils can have an increased oxygen demand, which leads to anaerobic conditions in soils with low oxygen permeability. Microbial degradation rates are extremely slow under anaerobic conditions, leading to longer oil persistence and effects.

D.4.f.2 Indicators of Exposure

Exposure can be documented through both visual and chemical methods. Visual observations of the presence of oil are most important during the early phases of the discharge, whereas chemical measures are valuable for documenting chronic and low-level exposures. Indicators of exposure are listed below:

Indicator of Exposure	**Measurement Methods**
Extent and degree of oil contamination and trampling on vegetation and soils	Aerial and ground surveys to make systematic, visual estimates of the areal extent and degree of oil adhering to vegetation and on/penetrated into soils using standardized terminology (Owens and Sergy, 1994); photographic or video documentation of visual observations; sampling of oiled soils and vegetation to fingerprint the oil and characterize oil weathering; summary statistics on the total acreage of each habitat by degree of oiling and trampling categories.
Soil contamination and toxicity	Collection of soil samples for chemical analysis to measure the levels of petroleum hydrocarbons present and toxicity.

D.4.f.3 Injury

Injury assessment studies of past incidents have concentrated on injury to vegetation. The objective is to quantify the injury in terms of reductions in the key measures of vegetation productivity and function and the areal extent and duration of the injury. These reductions can be translated into lost services and functions for valuable and sentinel species. Standard field methods for plant ecology studies can be used (e.g., Barbour et al., 1980). There have been many field studies of the effects of air pollution on vegetation that can be modified for oil pollution studies (e.g., Heck and Brandt, 1977).

Percent Live, Dead, and Stressed Vegetation. To quantify vegetation injury, estimates of the percent live, dead, and stressed vegetation can be made along transects utilizing a line-intercept sampling method. Transects are preferred because they provide topographic control. Using fixed transects allows better control for long-term monitoring of changes in cover. Alternately, study plots can be located in areas defined by degree of oiling and randomly located quadrats within each plot can then be used for making observations. Depending on the habitat, plant cover may need to be measured in three layers: canopy, understory, and herbaceous cover. Photography is important for documenting and supporting visual estimates or observations. Hemispheric photography and automated scanning of photographs can be used to determine percent canopy coverage (Anderson, 1964). Types of vegetation stress to be recorded include chlorosis, bronzing, marginal necrosis, leaf wilt, and leaf death. Ground stations can be used to verify estimates of vegetation die-back or stress measured from time-series aerial photography, using false-color infrared film (Murtha, 1978).

Above-ground Biomass. Net above-ground effects on production of herbaceous vegetation can be conducted by harvesting the vegetation from selected quadrats (subdivided into sections by degree of oiling) within the affected areas.

Growth. These measures may be valuable when particular species known to have high sensitivity to oil are present in the plant community. Under conditions of severe injury, each age class for key species can be studied using standard tree boring techniques, the diameter at breast height (dbh), and height measurements. These data can be used to calculate the time required for recovery to the pre-incident age structure in the affected area.

Seed Germination Success. For many species, stress is manifest as a reduction in reproduction. Comparisons between comparable oiled and unoiled study areas can be made of the percent of plants flowering and producing seeds, and seed viability. Seed germination studies can be conducted to determine the continued toxicity of soils and reduction in reproductive capability.

Net Erosion. Loss of vegetation could result in increased erosion, by wind or water. Sequential ground photography can be used to document sediment erosion following vegetation die-back. Seldom is erosion severe enough to detect using standard aerial photography. Erosion of stream banks can be monitored using standard topographic survey methods.

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